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Antifungal test of Piper betle linn leaf 35% on *Candida albicans*

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ABSTRACT

***Background**: There are a lot of traditional medicine that can be used as alternative medicine. One of them is a piper betle linn leaf which is proven as a non allergenic antibacterial agent if applied topically to the ulcers of the oral cavity. This makes 35% extract of Piper betle leaf as a potential material ulcer therapy in the oral cavity. This study compared antifungals test against Candida albicans which was given 35% extract of Piper betle linn leaf and 0.2% Chlorhexidine. **Purpose**: This study observed antifungal effectivity of 35% Piper betle linn leaf compared with 0.2% Chlorhexidine (positive control) and the negative control (untreated) against Candida albicans. This study was laboratory experiment. **Method**: The research used post test only design with completely randomized design. The research sample was a culture of Candida albicans that was given three treatments: The extract of Piper betle linn leaf, 0.2% Chlorhexidine (positive control) and the negative control (untreated). Furthermore, replicating the formula 9 times based on Federer theory and using the diffusion method as antifungal test. **Result**: The result showed an inhibitory zone of 35% Piper betle linn leaf extract smaller than the inhibitory zone of 0.2% Chlorhexidine (positive control), but there was the significant difference. **Conclusion**: The extract of 35% Piper betle linn leaf have antifungal activity against Candida albicans due to the phenol content in it.*

***Key words:** Antifungal test, 35% Piper betle linn leaf extract, diffusion method*

INTRODUCTION

Candida albicans is the most common species in candidiasis which is 70-80%. Candida albicans is a commensal organism present in the oral cavity in almost 50% of the population. However, in an appropriate circumstances Candida albicans can be pathogenic and form oral candidiasis. A research encountered 63.1% of adults are asymptomatic career of Candida albicans and 43.7% of children identified as asymptomatic career of Candida albicans with higher percentage (64%).[1]

Oral candidiasis can be treated with topical antifungal agents (eg, oral suspension nystatin, clotrimazole, amphotericin B) or Azole drugs group (eg, fluconazole, intraconazol). Azole drugs groups that available at this time are imidazole (eg, clotrimazole, miconazole, econazole, ketoconazole) and triazol (eg, flikonazol, itraconazole). The antifungal Azole drugs are effective, but there are reports that stating increasing numbers of resistance. Resistance of Candida albicans to azole drugs of different groups in a single treatment with azole derivatives are also reported in the literature. Diazol (eg ketoconazole, miconazole) are tended to have greater side effects. There is no azole drugs groups are completely safe. Hepatotoxicity may occur with

this drug, and potential endocrine toxicity may occur, especially if used in high doses. In addition, the azole class of drugs is too expensive.[2]

Chlorhexidine is an antiseptic and disinfectant approved as a mouthrinses by the Food and Drugs Administration (FDA) in 1980 which has a high effectivity to kills gram-negative, gram positive, tubercle, bacili, spores and some viruses. In Europe, 0.2% Chlorhexidine mouthrinses is used since 1970. Gluconat chlorhexidine 0.2% which is a cationic agent, biocide that has broad-spectrum activity against organisms are bactericidal and fungicidal.[3,4]

There are a lot of research in the development of traditional medicine that can be used as alternative medicine, because the material is easily obtained and affordable.[5] The piper betle linn leaf is a medicine plant with many benefits and all the parts containing antiseptic substances. Piper betle linn leaf are widely used by peoples to treat bleeding nose, itchy eyes, sore wound, bad breath, bleeding gums, and mouth sores (oral ulcers).Research laboratories by Supartinah, Suwondo and Yulianingsih showed that the piper betle linn leaf mouthrinses can kill a concentration of 25% of dental plaque bacteria, while the research by Suprihati, Suprapto and Lukitasari showed that mouthrinses of piper betle linn leaf in concentration of 25% for 30 seconds can kill Streptococcus sanguis.[6]

The results showed that mouthrinses of the piper betle linn leaf extract with concentration of 35% could inhibit oral Streptococcus viridians compared to 10% povidone iodine.[7] Siswanto[6] showed that the piper betle linn leaf extract ointment with concentration 35% are optimal to accelerate the wound healing process in the cheek mucosa of white rats when compared with concentrations of 15% and 25%.[7,8]

In vivo research in female mice that were given dosage effervescent tablets containing the piper betle linn leaf which highest toxic dose did not cause organ damage or death in test animals.[9] The research about the usage of the piper betle linn leaf decoction as a denture cleaning agent was able to inhibite the growth of Candida albicans although its inhibitory power did not as high as Chlorhexidine.[10]

Piper betle linn leaf extract with antifungal activity for oral cavity is already sold commercially in the form of toothpaste and mouthrinses to protect the gingival and dental health. Based on the description above, it is necessary to research more about the benefits of piper betle linn leaf as a natural antimicrobial material which can be used as an oral therapeutic agent.

Clinical trials were conducted on a 35% piper betle linn leaf extract as one of the active ingredient for the therapy of the oral cavity, the sensitivity test of the piper betle linn leaf extract 35% must be conducted and compared with 0.2% Chlorhexidine containing an antifungal effect against Candida albicans. The sensitivity tests are performed to determine the sensitivity of pathogenic microbials against antimicrobials. The most commonly used diffusion method is the agar diffusion method. The greater the inhibitory zone around the drug, the more sensitive the bacteria to the drug.

The purpose of this study was to observe the differences of the sensitivity of Candida albicans to the 35% piper betle linn leaf extract and 0.2% Chlorhexidine.

MATERIALS AND METHODS

The piper betle linn leaf of Java comes from Balai Materia Medica Batu, and then extracted in the Phytochemistry laboratory of UPT Balai Materia Medica Batu. The procedures were: Fresh piper betle linn leaf was separated from stems and washed clean and dried for ± 3 days. Furthermore, it was weighed and finely milled until produced dry powder. Then, weighing the piper betle linn leaf powder which will be extracted. The materials is used 75.524 g of powdered piper betle linn leaf (was inserted in 6 test tubes). Then inserted piper betle linn leaf powder into the tube 6 and given 95% ethanol solvent until 1020 ml. The extraction unit was programmed 5 times of extraction cycles with drying program for 60 minutes, the results are pure piper betle linn leaf extract 100% as much as 155 ml. The results of 100% pure extract plus sterile aquades diluted up to 35%.

The research was an laboratory experimental that was conducted with post test only design with completely randomized design. The research sample was a culture of Candida albicans taken at 37^0 C in 24 hours in BHI medium. Candida albicans colonies were taken with a sterile loop eye and cultured in Saborraud agar medium in petridish with a zigzag streaks. Then, Saborraud agar containing Candida albicans were divided into 3 groups, were given filter paper previously soaked in the 35% piper betle linn leaf extract (treatment) for 3 hours, were given the filter paper previously soaked with 0.2% Chlorhexidine (positive control) for 3 hours, and the group were given sterile filter paper; not given anything (negative control). The

results between the treatment groups and positive control will be compared to its diameter of inhibitory zone after incubation for 1 day.

RESULT

Based on observation and calculation the diameter of inhibitory zone of Candida albicans among groups that used the 35% piper betle linn leaf extract and 0.2% Clorhexidine with each of 9 samples was obtained the results presented in the form of tables and figures (Table 1 and figure 1)

Table 1. Diameter mean and standar deviation of inhibitory zone of *Candida albicans* in both of research groups.

	n	Minimum	Maximum	Mean	Std. Deviation
Ekstrak daun sirih 35%	9	7.00	10.00	8.2222	1.09291
Clorhexidine 0,2%	9	14.00	20.00	16.5556	2.00693
Negative	9	.00	.00	.0000	.00000
Valid N (listwise)	9				

Table 1 describes the diameter mean of inhibitory zone of Candida albicans that used the 35% piper betle linn leaf extract is 8.22 mm lower than 0.2% Clorhexidine is 16.56 mm.

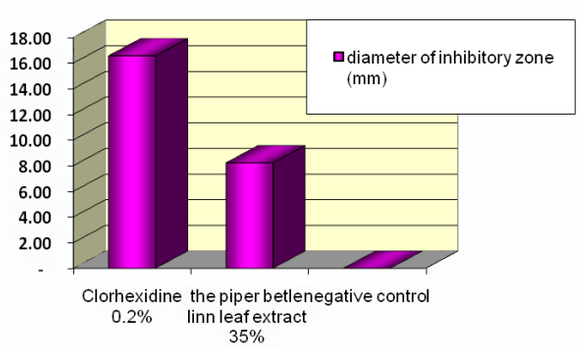

Figure 1. Diameter mean of inhibitory zone (mm) of *Candida albicans to each of the research groups.*

Figure 1 describes the diameter mean of inhibitory zone of Candida albicans which is lower on the usage of the 35% piper betle linn leaf extract compared with 0.2% Chlorhexidine.

Before the test and analysis of research groups is held, the normality tests was conducted on each group using the Kolmogorov Smirnov and all of the research group has a value greater than 0.05 ($p > 0.05$) which mean the data on the entire normally distributed research group, and it was continued using of different test parametric Independent t-tests to see the significance of differences between the resarch groups.

Independent t-tests was conducted to see the comparison of inhibitory zone diameter of Candida albicans between groups that used the 35% piper betle linn leaf extract with the group used 0.2% Chlorhexidine, was obtained significant differences in inhibitory zone diameter of Candida albicans between the groups using the 35% piper betle linn leaf extract and the groups using 0.2% Chlorhexidine, it was seen from the significance of differences in the Independent T-test smaller than 0.05 ($p = 0.000$ or $p < 0.05$).

DISCUSSION

The sensitivity test to Candida albicans was conducted using diffusion method. It aimed to know the size of inhibitory zone diameters of the 35% piper betle linn leaf extract if compared with 0.2% Chlorhexidine. The larger the diameter of inhibitory zone indicates the higher its antifungal trait. Candida albicans is more sensitive to 0.2% Chlorhexidine rather than the 35% piper betle linn leaf extract, this means that the antifungal trait to component phenols in the essential oil of the piper betle linn leaf is not as big 0.2% Chlorhexidine.

The essential oils in piper betle linn leaf consist of phenol components (phenol propenyl) of 60% and the rest is non-phenol. Phenol is antiseptic component which consists of eugenol, estragol, chavibetol (betel phenol 9), and chavikol which can kill some gram positive and gram negative bacteria.[11] Essential oils can also be used as an anti-fungal based on research conducted by Ali Yusran[12], especially to cure candida infections in the oral cavity.[13]

Antifungal traits of piper betle linn leaf is originated from kavicol and essential oil that able to kill fungi by protein denaturation process. This is to prevent the occurence of infection that could inhibit the healing process.[8,11,14] This is what makes the Candida albicans sensitive to the 35% piper betle linn leaf extract although not as much as 0.2% Chlorhexidine.

Selection of piper betle linn leaf needs to be held to obtain high power antifungals. The use of the piper betle linn leaf in young leaves are better than the old one, because levels of essential oil in young

leaf is higher if compared the older leaves.[15] Another consideration is selecting leaves that are still fresh and dried, bright colored, perfect shape, free of disease (fungus or pests), and does not change in color.[16]

The research used 95% ethanol solvent. According to the research of Soemarno[17] in the piper betle linn leaf extraction process should be used an organic solvent such as ether, alcohol, and chloroform. This is because essential oils are insoluble in water solvent.

Chlorhexidine can be used as an antiseptic mouthrinses on the initial treatment of candidiasis or as a prophylactic standard to help control recurrent infections. Chlorhexidine has an antifungal spectrum and can suppress the colonization.[18] Inside fungi, chlorhexidine penetrate into the cell wall or plasma membrane and cytoplasm, resulting in semipermeable membrane damage followed by the leakage of intracellular contents that ends with cell death. Chlorhexidine has a biphasic effect that affects the lysis of protoplasm, but this condition has decreased at higher concentrations (> 25 mg/ml). Fungal cell walls have a certain amount of change in the presence of chlorhexidine. Effective concentration for the use of chlorhexidine as a mouthrinses was 0.12% and 0.25% 10 ml 2 times a day.[19]

It can be concluded that 35% piper betle linn leaf extract have antifungal inhibitory power against Candida albicans due to the phenol content in it.

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